Research Article

Cancer Epidemiology, Biomarkers & Prevention

Dietary Intake of One-Carbon Metabolism–Related Nutrients and Pancreatic Cancer Risk: The Singapore Chinese Health Study

Joyce Y. Huang[1,2], Lesley M. Butler[1,2], Renwei Wang[1], Aizhen Jin[3], Woon-Puay Koh[4,5], and Jian-Min Yuan[1,2]

Abstract

Background: Nutrients involved in one-carbon metabolism are hypothesized to protect against pancreatic cancer development.

Methods: The Singapore Chinese Health Study database was used to prospectively examine the association between intake of one-carbon metabolism–related nutrients and pancreatic cancer risk. Between 1993 and 1998, 63,257 men and women ages 45 to 74 years were enrolled into the cohort. The daily intakes of the following one-carbon metabolism–related nutrients were assessed at enrollment using a 165-item food frequency questionnaire: betaine, choline, folate, and vitamins B_2, B_6, and B_{12}. Multivariable HRs and 95% confidence intervals (CI) for pancreatic cancer risk associated with dietary intakes of one-carbon metabolism–related nutrients were calculated.

Results: As of December 2013, 271 incident pancreatic cancer cases were identified during an average of 16.3 years of follow-up. Higher intakes of vitamin B_6 and choline were associated with statistically significant decreases in the risk of developing pancreatic cancer. Compared with the lowest quartile, HRs (95% CIs) for the highest quartiles of vitamin B_6 and choline were 0.52 (0.36–0.74; P trend = 0.001) and 0.67 (0.48–0.93; P trend = 0.04), respectively. There were no clear associations between the other one-carbon metabolism–related nutrients and pancreatic cancer risk.

Conclusion: Our study suggests that higher intake of vitamin B_6 and choline may lower the risk of pancreatic cancer.

Impact: Our prospective findings are consistent with the *in vivo* evidence for protective roles of vitamin B_6 and choline on pancreatic cancer development. *Cancer Epidemiol Biomarkers Prev; 25(2); 417–24. ©2015 AACR.*

Introduction

Pancreatic cancer is among the most deadly malignancies in the world. In the U.S., pancreatic cancer is the fourth leading cause of cancer-related death in both men and women (1). In 2014, it is estimated that 20,170 men and 19,420 women died from pancreatic cancer (1). The median survival of pancreatic cancer is only 3 to 6 months, due in part to the lack of effective treatments (2). Cigarette smoking and obesity are the only established modifiable risk factors for pancreatic cancer (3, 4). The identification of novel primary prevention targets is a viable approach for reducing the burden of pancreatic cancer.

One-carbon metabolism is a set of interconnected pathways that supply methyl groups for DNA synthesis, repair, and methylation (5). Adequate DNA methylation maintains chromosome stability and prevents gene disruption (6). Studies using a global methylation profiling approach showed that a substantial number of genes were aberrantly methylated not just in advanced pancreatic cancers (7), but also in precancerous lesions (8), indicating a potential time window when chemoprevention agents that target DNA methylation pathways could have a beneficial impact. Diet is a major source for key substrates and co-factors involved in one-carbon metabolism, such as vitamin B_6, choline, and folate (9). Vitamin B_6 is a co-factor for multiple enzymes in the one-carbon metabolism pathway, including serine hydroxymethyltransferase for nucleotide synthesis (10) and remethylation of homocysteine (11), and cystathionine β-synthase and cystathione γ-lyase for generation of glutathione (12). In rodents, diets deficient in methyl donors (i.e., choline, methionine, and/or folate) resulted in global hypomethylation (13–15) and increased incidence of neoplastic lesions induced by carcinogens in the pancreas (16–18). Therefore, low dietary intake of these nutrients may interfere with the normal function of one-carbon metabolism pathways (19, 20), and thus increase the risk of developing pancreatic cancer.

A majority of epidemiologic studies that have evaluated one-carbon metabolism–related nutrients in relation to pancreatic cancer risk have focused on the potential role of folate. A recent pooled analysis of 14 prospective cohort studies conducted mostly in U.S. populations, reported that dietary folate was not associated with pancreatic cancer risk (21). Similarly, there was no trend with increasing blood folate levels and pancreatic cancer risk in the European Investigation of Cancer cohort study (22) or in a pooled analysis of U.S. cohorts (23).

[1]Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania. [2]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania. [3]National Registry of Diseases Office, Health Promotion Board, Singapore. [4]Duke-NUS Graduate Medical School Singapore, Singapore. [5]Saw Swee Hock School of Public Health, National University of Singapore, Singapore.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Lesley M. Butler, University of Pittsburgh Cancer Institute, UPMC Cancer Pavilion, 5150 Centre Avenue–Suite 4C, Pittsburgh, PA 15232. Phone: 412-623-3386; Fax: 412-864-7838; E-mail: butlerl3@upmc.edu

doi: 10.1158/1055-9965.EPI-15-0594

Few prospective studies of pancreatic cancer have considered the potential role of one-carbon metabolism–related nutrients other than folate. Dietary methionine was strongly associated with lower risk of pancreatic cancer in a Swedish study (24), but not in a cohort of Finnish male smokers (25). Although neither cohort supported dietary vitamin B_6 as a possible protective factor for pancreatic cancer (24, 25), a recent report showed a statistically significant inverse association for plasma pyridoxal phosphate (PLP), the bioactive form of vitamin B_6, in European women (22). A large population-based case–control study in the U.S. reported a positive association and trend with increasing pancreatic cancer risk for dietary vitamin B_{12} (26). There have been no epidemiologic studies of other one-carbon related nutrients, such as choline or betaine and pancreatic cancer risk.

Due to the involvement of multiple nutrients and the complexity of one-carbon metabolism pathways, a comprehensive assessment of the nutrients involved and their associations with pancreatic cancer risk is needed. Therefore, it is worthwhile to investigate these associations in a prospective cohort with well-characterized dietary intake. We analyzed data from a prospective cohort of Singapore Chinese to test the hypothesis that higher intake of one-carbon metabolism–related nutrients are inversely associated with the risk of developing pancreatic cancer.

Materials and Methods

Study design and population

The design of Singapore Chinese Health Study has been previously described in detail (27). Briefly, between 1993 and 1998, 63,257 Chinese men and women between ages of 45 and 74 years living in Singapore were enrolled into the cohort study. The cohort was drawn from residents of government-built housing estates (86% of all Singaporeans during the recruitment time period). Study subjects were restricted to two major dialect groups of Chinese in Singapore (Hokkien and Cantonese). The study was approved by the Institutional Review Boards of the National University of Singapore and the University of Pittsburgh.

Assessment of one-carbon metabolism–related nutrients

At the time of recruitment, each participant completed an in-person interview with a structured questionnaire asking for information on demographics, uses of tobacco and alcohol, medical history, and physical activity. To assess usual dietary intake, a 165-item semi-quantitative food frequency questionnaire (FFQ) was used. The FFQ was developed for the target study population and validated using a series of 24-hour recall interviews and repeated administration of the FFQ in a subpopulation of the cohort (28).

Average daily intake of one-carbon metabolism–related nutrients was calculated by multiplying the usual frequency and portion size of each food item by the nutrient content using the food composition values from the Singapore Food Composition Database. The original Singapore Food Composition Database contained the levels of 98 nutritive/nonnutritive food components, including folate, and vitamins B_2, B_6, and B_{12}, per 100 g of edible food for each food item included in the FFQ (28). The nutrient content information of betaine (29–34), choline (30, 32, 34), and methionine (32, 34) has recently become available and has been added into the Singapore Food Composition Database based on the data published by the U.S. Department of Agriculture and the University of Minnesota's Nutrition Coordinating Center Food and Nutrient Database.

Our team has previously reported the correlation coefficients for total calorie intake and selected nutrients (including folate) from the FFQ versus the 24-hour recalls information collected from 858 randomly chosen cohort members for the purpose of validating the FFQ (28). The correlation coefficient ranged from 0.31 to 0.53 for total calories and 0.50 to 0.69 for calorie-adjusted folate by residual method (35) across sex and dialect groups (Cantonese and Hokkien; ref. 28).

Ascertainment of pancreatic cancer cases

Incident pancreatic cancer cases (International Classification of Diseases-Oncology codes, 2nd edition, C25) were identified through record linkage with the databases of the nationwide Singapore Cancer Registry and the Singapore Registry of Births and Deaths. The national cancer registry has been in place since 1968 and has been shown to be comprehensive in recording cancer cases among the entire population (36). As of December 31, 2013, only 57 participants from this cohort were known to be lost to follow-up because of migration out of Singapore ($n = 30$) or for other reasons ($n = 27$). Among those under follow-up, 276 cohort members who were free of cancer at baseline developed pancreatic cancer.

Statistical analysis

Person-years of follow-up time was counted from the date of baseline interview to the diagnosis of pancreatic cancer, lost to follow-up, death of any cause, or December 31, 2013, whichever occurred first. Among the cohort participants, 1,936 had a history of cancer before enrollment, and thus were excluded from the present analysis. Another 459 men and 564 women were excluded due to extreme values of total calorie intake (men: <700 or >3,700 kcal/day, women: <600 or >3,000 kcal/day). In total, 60,298 subjects including 271 pancreatic cancer cases were included in the analysis.

Nutrient variables were presented as the values adjusted for total calorie intake by using the residual method (35). Cox proportional hazards regression method (37) was used to estimate the HRs and 95% confidence intervals (CI) for the associations between one-carbon metabolism–related nutrients and pancreatic cancer risk. Study subjects were grouped into quartiles based on the distribution of nutrient intake in the entire cohort. The nutrients were coded as ordinal values (1, 2, 3, and 4) of quartile variables to assess the linear trends of the nutrient–pancreatic cancer association. We did not identify any violation of the proportional hazard assumption.

On the basis of previous analyses in the cohort, we controlled for age at baseline (years), sex, year of baseline interview (1993–1995, 1996–1998), dialect group (Cantonese and Hokkien), and the level of education (no formal schooling, primary school, and secondary school or above) in all multivariable models. We further adjusted for body mass index (BMI; <18.5, 18.5–21.4, 21.5–24.4, 24.5–27.4, $\geq$27.5 kg/m^2; refs. 38, 39), smoking status (never smokers, former smokers, and current smokers), alcohol drinking (nondrinker and drinker), weekly use of vitamin/mineral supplement (no and yes), self-reported physician diagnosed diabetes (no and yes), any weekly physical activity (no and yes), and total daily caloric intake (tertiles).

Covariates were included in the final multivariable regression models, because including the variable in the base model resulted in change of 10% or more in the HR for pancreatic cancer and at least one of the one-carbon metabolism–related nutrients, or the variable had been previously reported to be associated with pancreatic cancer in the present study population or other populations.

We further performed stratified analyses by sex. To rule out potential effects of subclinical pancreatic cancer on intake of one-carbon metabolism–related nutrients we performed secondary analyses after excluding pancreatic cancer cases and person-years during the first 2 years of follow-up after enrollment. Statistical analyses were conducted using the SAS version 9.3 (SAS Institute, Inc.). All P values were two-sided and considered statistically significant if less than 0.05.

Results

After an average 16.3 years of follow-up, 271 cohort members (138 males and 133 females) developed pancreatic cancer. The mean age at cancer diagnosis was 72.0 years for males, and 71.6 years for females. The mean time from baseline interview to cancer diagnosis was 10.5 years (range, 3 months to 20.2 years).

Baseline characteristics were evaluated by highest and lowest quartile intake levels of vitamin B_6 and choline, two of the one-carbon metabolism–related nutrients with *a priori* hypothesis for associations with pancreatic cancer risk (Table 1). Overall, men (vs. women) were more likely to be ever smokers (57.7% vs. 8.7%) and alcohol drinkers (31.4% vs. 9.0%). Men and women in the highest quartile of vitamin B_6 intake were more likely to have a higher BMI and to be a never smoker than those in the lowest quartile. Among men, alcohol drinking and diabetes were more prevalent in the highest quartile of vitamin B_6. Men in the highest quartile of choline intake were more likely to be smokers and alcohol drinkers compared with those in the lowest quartile.

Positive correlations were observed between a majority of the one-carbon metabolism–related nutrients (Supplementary Table S1). The strongest correlations were observed between choline and other nutrients, including methionine [Spearman correlation coefficient (r) = 0.70], vitamin B_2 ($r = 0.60$), and B_{12} ($r = 0.70$). The weakest correlations were observed between methionine and other nutrients, including betaine ($r = 0.00$) and folate ($r = 0.05$). The Spearman correlation coefficient of vitamin B_6 and choline was 0.50.

Risk of pancreatic cancer in females was 30% lower than that in males (Table 2). Compared with never smokers, no

Table 1. Participant characteristics according to intake of vitamin B_6 (mg) and choline (mg) at baseline, the Singapore Chinese Health Study, 1993–2013

	Men				Women			
	Vitamin B_6 intake[a]		Choline intake[a]		Vitamin B_6 intake[a]		Choline intake[a]	
Characteristics	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4
N	8,244	6,953	8,512	6,680	6,956	8,130	15,001	15,012
Mean age, y	56.8	56.2	56.6	55.9	57.7	54.8	57.7	54.6
Body mass index (kg/m^2), %								
<18.5	7.8	5.5	6.4	7.3	6.8	5.2	5.9	5.8
18.5–21.4	24.7	21.8	23.2	23.1	22.2	22.0	22.1	22.3
21.5–24.4	45.0	42.8	45.4	41.8	47.8	44.3	48.2	43.2
24.5–27.4	16.0	20.7	17.6	18.5	15.0	18.2	15.5	18.5
≥27.5	6.6	9.3	7.4	9.3	8.2	10.2	8.2	10.2
Education, %								
No formal education	12.7	8.1	11.4	9.1	48.8	30.0	48.1	31.1
Primary school	55.0	46.5	53.4	48.7	38.2	40.0	38.0	40.2
≥Secondary school	32.3	45.4	35.2	42.2	13.0	30.1	13.9	28.7
Smoking status, %								
Never smokers	39.2	44.7	44.6	37.5	88.2	93.9	90.3	91.6
Former smokers	21.1	22.2	22.3	20.2	3.2	2.1	3.0	2.4
Current smokers	39.7	33.1	33.1	42.3	8.6	4.0	6.8	6.1
Alcohol drinking, %								
Nondrinkers	74.5	61.0	74.6	57.9	90.6	90.1	92.1	88.4
<7 drinks/week	20.5	23.1	21.1	24.2	7.7	8.6	6.7	10.1
≥7 drinks/week	5.0	15.9	4.4	17.9	1.7	1.4	1.2	1.6
Weekly vitamin/minerals use (% Yes)	3.9	6.5	4.1	5.9	5.7	10.5	5.6	9.5
Weekly physical activity (% Yes)	42.1	46.9	44.6	41.7	21.3	30.1	22.6	27.5
Diabetes (% Yes)	6.9	9.5	6.8	10.3	8.4	8.2	8.0	10.1
Mean calorie intake, kcal/day	1,879.8	1,809.2	1,878.6	1,827.3	1,540.1	1,486.2	1,415.8	1,645.8
Mean nutrient intake[b]								
Betaine, mg/day	63.4	81.4	62.8	79.3	71.1	76.6	69.5	74.9
Choline, mg/day	188.7	259.8	160.4	306.0	199.7	260.8	174.3	292.4
Folate, μg/day	119.2	185.3	127.8	169.2	129.6	194.2	138.0	178.8
Methionine, mg/day	1,171.2	1,400.6	1,098.0	1,540.6	1,208.5	1,471.7	1,129.8	1,597.5
Vitamin B_2, mg/day	0.8	1.0	0.7	1.1	0.9	1.1	0.8	1.1
Vitamin B_6, mg/day	0.8	1.4	1.0	1.2	0.9	1.4	1.0	1.2
Vitamin B_{12}, μg/day	1.8	2.6	1.5	3.1	2.0	2.7	1.6	3.1

Abbreviations: Q1, 1st quartile; Q4, 4th quartile.

[a]Nutrient intake was adjusted for daily total calorie intake by residual method. Quartiles of vitamin B_6 and choline were based on the distribution among the entire cohort.

[b]Nutrient intake was adjusted for daily total calorie intake by residual method.

Table 2. Associations between potential risk factors and pancreatic cancer risk overall, and stratified by sex, the Singapore Chinese Health Study, 1993–2013

	Total subjects		Men		Women	
Characteristics	**Cases, *N***	**HR (95% CI)[a]**	**Cases, *N***	**HR (95% CI)[a]**	**Cases, *N***	**HR (95% CI)[a]**
Age	271	1.09 (1.08–1.11)	138	1.10 (1.07–1.12)	133	1.09 (1.06–1.12)
Female vs. male	271	0.70 (0.55–0.89)	138	—	133	—
Body mass index, kg/m^2						
<18.5	22	1.44 (0.91–2.27)	13	1.53 (0.84–2.79)	9	1.33 (0.66–2.69)
18.5–21.4	62	1.18 (0.86–1.60)	37	1.27 (0.84–1.92)	25	1.06 (0.66–1.70)
21.5–24.4	114	1.00 (ref)	57	1.00 (ref)	57	1.00 (ref)
24.5–27.4	55	1.33 (0.97–1.84)	26	1.20 (0.75–1.90)	29	1.48 (0.95–2.32)
≥27.5	18	0.93 (0.56–1.52)	5	0.58 (0.23–1.45)	13	1.21 (0.66–2.21)
Education						
No formal education	82	1.00 (ref)	14	1.00 (ref)	68	1.00 (ref)
Primary school	121	1.07 (0.78–1.45)	75	1.30 (0.73–2.32)	46	1.00 (0.68–1.49)
≥Secondary school	68	1.16 (0.81–1.68)	49	1.45 (0.79–2.67)	19	1.02 (0.60–1.76)
Smoking						
Never smokers	166	1.00 (ref)	54	1.00 (ref)	112	1.00 (ref)
Former smokers	34	0.96 (0.64–1.45)	31	0.92 (0.59–1.44)	3	0.76 (0.24–2.41)
Current smokers	71	1.40 (1.02–1.92)	53	1.24 (0.84–1.81)	18	1.93 (1.17–3.20)
Alcohol drinking						
Nondrinkers	222	1.00 (ref)	105	1.00 (ref)	117	1.00 (ref)
<7 drinks/week	39	1.00 (0.71–1.42)	24	0.77 (0.49–1.20)	15	1.68 (0.98–2.89)[b]
≥7 drinks/week	10	0.80 (0.42–1.52)	9	0.79 (0.40–1.56)	1	—
Diabetes						
No	241	1.00 (ref)	122	1.00 (ref)	119	1.00 (ref)
Yes	30	1.30 (0.88–1.90)	16	1.49 (0.88–2.52)	14	1.19 (0.64–1.95)
Weekly vitamin/mineral supplement use						
No	256	1.00 (ref)	132	1.00 (ref)	124	1.00 (ref)
Yes	15	0.89 (0.53–1.51)	6	0.80 (0.35–1.82)	9	0.99 (0.50–1.94)

[a]HR and 95% CI were adjusted for age (years), sex, father's dialect group (Cantonese, Hokkien), and year of interview (1993–1995, 1996–1998).
[b]HR and 95% CI were calculated for drinkers vs. nondrinkers. Only one case was in women ≥7 drinks/week, and thus <7 drinks/week was combined with ≥7 drinks/week.

association was observed with former smokers, regardless of year since quitting (data not shown). Current smokers who smoked for 30 or more years had an increased pancreatic cancer risk (HR = 1.61; 95% CI, 1.19–2.16) that was similar to those who smoked for less than 30 years (HR = 1.55; 95% CI, 0.84–2.88), compared with never smokers. Among women, current smokers experienced a statistically significant 93% increased risk of pancreatic cancer compared with never smokers, and alcohol consumption of one or more drinks per week was associated with statistically nonsignificant 68% increased risk of pancreatic cancer, compared with nondrinkers. There were no significant associations between smoking or drinking and risk of pancreatic cancer in men.

Higher intakes of choline and vitamin B_6 were associated with decreased risk of pancreatic cancer in a dose-dependent manner (Table 3). The inverse associations between dietary choline and vitamin B_6 and pancreatic cancer risk remained after excluding the first 2 years of follow-up. Comparing the highest versus the lowest quartile, the HRs (95% CIs) for choline and vitamin B_6 were 0.64 (0.45–0.90) and 0.54 (0.37–0.78), respectively (both P's for trend ≤ 0.02). There was no association for pancreatic cancer risk with the intake of betaine, folate, methionine, vitamins B_2, or B_{12} (Table 3). For comparison with a previous reports (24, 26), we evaluated potential joint effects between dietary folate and methionine on pancreatic cancer risk. Although no clear pattern emerged, higher dietary methionine was associated with reduced risk when folate was low (HR = 0.64; 95% CI, 0.42–0.99; comparing third to first tertile; Supplementary Table S2). There was no evidence for joint effects on pancreatic cancer risk with folate and vitamin B_6 or choline, or with vitamin B_6 and choline (all P's for interaction > 0.6).

The association between dietary choline or vitamin B_6 and pancreatic cancer risk was more apparent in men than in women (Table 3). However, we did not detect a statistically significant interaction between gender and either choline or vitamin B_6 on pancreatic risk (both P's ≥ 0.15). There were also dose-dependent inverse associations for pancreatic cancer risk only in men for methionine (P for trend = 0.02) and vitamin B_{12} (P for trend = 0.047). The gender-nutrient interaction on pancreatic cancer risk was statistically significant for vitamin B_{12} (P for interaction = 0.01), but not for methionine (P for interaction = 0.11).

Discussion

We prospectively evaluated whether one-carbon metabolism–related nutrients were associated with pancreatic cancer risk and found that dietary intake of choline and vitamin B_6 demonstrated inverse, statistically significant trends. Highest quartiles, as compared with the lowest quartiles, of dietary vitamin B_6 and choline were associated with a 48% and 33% decrease in pancreatic cancer risk, respectively. We reported no association with the other one-carbon metabolism related-nutrients, including betaine, folate, methionine, vitamins B_2, and B_{12}.

Our finding of a dietary vitamin B_6-pancreatic cancer risk association differs from the null results observed in prospective cohorts in Sweden (24) and Finland (25), as well as a large population-based case–control study in the U.S. (26). Possible explanations for the discrepancy include the relatively low intake of vitamin B_6 in our cohort, as well as differences in the major food sources of vitamin B_6 in a Chinese versus Western diet. Less than 15% of our cohort met the U.S. Recommended Daily Allowance of 1.7 and 1.5 mg for men and women, respectively (9). In contrast, 75% of the Swedish cohort and 80% of the U.S. control

Table 3. Multivariate analysis of one-carbon metabolism dietary factors and pancreatic cancer incidence by sex, Singapore Chinese Health Study 1993–2013

		All		Men		Women	
Nutrients	**Median intake[a]**	**Cases, *N***	**HR (95% CI)[b]**	**Cases, *N***	**HR (95% CI)[b]**	**Cases, *N***	**HR (95% CI)[b]**
Betaine, mg/day							
Q1	41.56	68	1.00 (ref)	43	1.00 (ref)	25	1.00 (ref)
Q2	59.43	75	1.12 (0.81–1.57)	40	1.21 (0.78–1.87)	35	1.10 (0.65–1.83)
Q3	70.84	71	1.03 (0.73–1.44)	29	0.82 (0.51–1.33)	42	1.30 (0.79–2.14)
Q4	105.62	57	0.80 (0.56–1.14)	26	0.66 (0.40–1.08)	31	1.05 (0.62–1.79)
P-trend			0.19		0.051		0.71
Choline, mg/day							
Q1	176.30	97	1.00 (ref)	62	1.00 (ref)	35	1.00 (ref)
Q2	217.25	54	0.57 (0.40–0.80)	22	0.48 (0.29–0.78)	32	0.70 (0.43–1.13)
Q3	245.27	64	0.72 (0.52–1.00)	29	0.71 (0.45–1.11)	35	0.79 (0.49–1.27)
Q4	286.79	56	0.67 (0.48–0.93)	25	0.55 (0.34–0.88)	31	0.86 (0.53–1.41)
P-trend			0.04		0.02		0.69
Folate, μg/day							
Q1	108.24	74	1.00 (ref)	45	1.00 (ref)	29	1.00 (ref)
Q2	137.56	66	0.94 (0.67–1.32)	33	1.00 (0.63–1.58)	33	0.92 (0.55–1.52)
Q3	162.90	74	1.12 (0.81–1.56)	30	0.94 (0.58–1.50)	44	1.35 (0.83–2.17)
Q4	207.21	57	0.89 (0.63–1.28)	30	0.90 (0.56–1.44)	27	0.94 (0.54–1.61)
P-trend			0.82		0.62		0.73
Methionine, mg/day							
Q1	1,073.17	72	1.00 (ref)	47	1.00 (ref)	35	1.00 (ref)
Q2	1,268.95	88	1.26 (0.92–1.73)	48	1.35 (0.90–2.04)	40	1.17 (0.71–1.92)
Q3	1,414.90	58	0.88 (0.62–1.25)	20	0.62 (0.37–1.05)	38	1.14 (0.69–1.89)
Q4	1,625.25	53	0.82 (0.57–1.17)	23	0.67 (0.41–1.10)	30	1.02 (0.60–1.72)
P-trend			0.09		0.02		0.98
Vitamin B_2, mg/day							
Q1	0.71	71	1.00 (ref)	48	1.00 (ref)	23	1.00 (ref)
Q2	0.86	70	1.03 (0.74–1.44)	34	0.96 (0.61–1.51)	36	1.20 (0.71–2.04)
Q3	0.98	61	0.92 (0.65–1.30)	31	0.86 (0.54–1.36)	30	1.08 (0.63–1.87)
Q4	1.20	69	1.01 (0.72–1.42)	25	0.68 (0.42–1.11)	44	1.55 (0.93–2.59)
P-trend			0.88		0.12		0.13
Vitamin B_6, mg/day							
Q1	0.88	95	1.00 (ref)	62	1.00 (ref)	33	1.00 (ref)
Q2	1.02	64	0.71 (0.51–0.98)	22	0.48 (0.29–0.80)	42	1.03 (0.64–1.64)
Q3	1.13	68	0.80 (0.58–1.11)	31	0.73 (0.47–1.14)	37	0.98 (0.60–1.58)
Q4	1.33	44	0.52 (0.36–0.74)	23	0.45 (0.28–0.74)	21	0.66 (0.38–1.15)
P-trend			0.001		0.004		0.16
Vitamin B_{12}, μg/day							
Q1	1.43	74	1.00 (ref)	54	1.00 (ref)	20	1.00 (ref)
Q2	2.05	69	0.97 (0.69–1.35)	29	0.71 (0.45–1.12)	40	1.57 (0.91–2.70)
Q3	2.52	68	0.99 (0.70–1.38)	34	0.91 (0.59–1.41)	34	1.31 (0.75–2.28)
Q4	3.26	60	0.88 (0.62–1.24)	21	0.54 (0.33–0.90)	39	1.60 (0.93–2.74)
P-trend			0.51		0.047		0.21

[a]Nutrient intake levels were adjusted for daily total calorie intake using residual method.

[b]Adjusted for age (continuous, year), sex (male, female), year of interview (1993–1995, 1996–1998), dialect group (Cantonese, Hokkien), education (no formal education, primary school, and secondary school or higher), BMI (<18.5, 18.5–21.4, 21.5–24.4, 24.5–27.4, ≥27.5 kg/m^2), smoking status (never, former, current), diabetes (no, yes), alcohol drinking (no, yes), and weekly vitamin use (no, yes). Stratified analyses were not adjusted by sex. The cutoff values for one-carbon metabolism–related nutrients were the same for men and women.

population had consumed more than 1.7 mg/day from food only (24, 26). The major food sources of vitamin B_6 in our cohort were rice (25%) and fish (16%), compared with meat (29%) and cereals (17%) in Sweden (40), and read-to-eat cereal (13%), poultry (9.0%), and beef (8.7%) in the U.S. (41). The suggestive evidence that red meat intake is associated with an increased risk of pancreatic cancer (42) may partially explain the null results with vitamin B_6 that were observed in Western study populations. It is also possible that once the daily requirement is met there is no additional benefit with higher intake of vitamin B_6 on the risk of pancreatic cancer.

Our dietary vitamin B_6-pancreatic cancer finding supports the statistically significant inverse associations with higher circulating PLP, the bioactive form of vitamin B_6, and pancreatic cancer risk that were reported in two European studies (22, 43), but not the null finding from a pooled analysis of four U.S. cohorts (23). We have previously reported a modest statistically significant correlation with plasma PLP and dietary vitamin B_6 in a healthy subset of our study population ($r = 0.17$, $P = 0.0003$; ref. 44). In addition to the lower intake and different major food sources of vitamin B_6 in our study population, perhaps our FFQ also captured the internal dose more accurately, compared with the studies that reported no association with dietary vitamin B_6 and pancreatic cancer risk.

A protective effect of vitamin B_6 on pancreatic cancer development is biologically plausible given vitamin B_6's role as a co-factor for enzymes involved in the DNA synthesis and methylation pathways of one-carbon metabolism. As a co-factor for serine hydroxymethyltransferase, a diet low in vitamin B_6 results in a decreased production of the methyl donor, methylene-THF (45–47). A decrease in the methylene-THF pool may overload the DNA repair system by increasing uracil incorporation into DNA, and eventually lead to chromosome breaks (47, 48). Global DNA hypomethylation has been linked with genomic instability (49) and tumorigenesis (50, 51). The level of methylation of long interspersed nuclear element-1 (LINE-1) DNA sequences from peripheral lymphocytes is used as a biomarker for genomic DNA methylation status (52), and lower levels are associated with increased risk of some cancers (53). The level of LINE-1 methylation was measured in a healthy subset of our Singapore Chinese cohort (54), and in a secondary analysis, we found that dietary vitamin B_6 had a weak positive correlation with LINE-1 ($r = 0.12$, $P = 0.007$). In summary, it is biologically plausible that adequate intake of vitamin B_6 may reduce the risk of pancreatic cancer through its beneficial effects on DNA synthesis and methylation status.

Vitamin B_6 may play a role in preventing DNA from oxidative damage. In rats fed a vitamin B_6-deficient diet, decreased activity of pancreatic glutathione reductase, an enzyme that maintains the cellular glutathione level was reported (55). Glutathione is an antioxidant that is required for maintenance of the cellular redox state and detoxification of carcinogens, and low glutathione may impair the antioxidant defense system (12). Therefore, increasing oxidative stress may represent a mechanistic pathway by which low intake of vitamin B_6 may lead to increased pancreatic cancer risk.

To our knowledge, no epidemiological study has studied the relationship between dietary choline and pancreatic cancer risk. Our observed inverse association between choline intake and pancreatic cancer risk is consistent with the experimental evidence that dietary deficiencies of methyl donors, such as choline, led to aberrant differentiation and function of the exocrine pancreas and contributed to pancreatic carcinogenesis (56). A long-term choline-deficient ethionine (an antagonist of methionine)-supplemented diet in mice with induced chronic pancreatitis resulted in an increase in the expression of key molecules in the pancreatic carcinogenesis process, such as EGFR, K-Ras, and TGFα (57). Furthermore, a choline-deficient diet was able to shorten the induction period and increase the incidence of carcinogen-induced pancreatic carcinomas in hamsters (16, 58). In summary, our observed inverse association between dietary choline and pancreatic cancer risk is biologically plausible, but it is not clear whether the role of choline is independent of the potential effects of methionine and/or vitamin B_{12}, given that intake of these three nutrients are strongly correlated with each other.

We did not observe statistically significant associations with dietary betaine, folate, methionine, vitamin B_2, or B_{12} for pancreatic cancer risk. Dietary intake of betaine or vitamin B_2 has not been previously evaluated in relation to pancreatic cancer risk. Our finding for no association with folate was consistent with a pooled analysis of 14 prospective cohort studies (21). Our finding for no association with vitamin B_{12} was consistent with results from the only other prospective study to evaluate an association with pancreatic cancer risk (25). Our finding for methionine was similar to two (25, 59), but not all (24) prospective studies that evaluated methionine–pancreatic cancer risk associations. In our sex-stratified results, a vitamin B_{12}–pancreatic cancer inverse association was only present in men, and vitamin B_{12} was the only nutrient with a statistically significant interaction with sex. Vitamin B_{12} functions as a co-factor for methionine synthase, an enzyme that converts homocysteine to methionine (9). In our data, vitamin B_{12} was strongly correlated with methionine and choline (Supplementary Table S1), making it difficult to tease out the individual effects of these three nutrients on pancreatic cancer risk. The inverse associations with vitamin B_{12}, methionine, and choline intake and pancreatic cancer risk among men in our study suggests that compared with women, men may be more susceptible to low intake of these one-carbon metabolism–related nutrients (60). Our sex-specific findings, however, should be interpreted with caution, as they may be due to chance given the small number of cases available in the stratified analyses.

The strengths of our study include a prospective design, long duration of follow-up, and a comprehensive assessment of one-carbon metabolism–related nutrients. There are also limitations of our study. Due to the nature of an observational study and the one-time assessment of diet, our results may be influenced by misclassification of usual diet during the long follow-up period. However, given the prospective design, the potential for misclassification is unlikely to be different in cases and noncase participants; the nondifferential misclassification could bias our results toward the null.

In summary, this prospective cohort study demonstrated statistically significant, inverse associations between dietary vitamin B_6 and choline, and pancreatic cancer risk. These novel findings support the hypotheses that vitamin B_6 and choline are relevant in pancreatic carcinogenesis. Future studies are needed to study the underlying mechanisms of how vitamin B_6 and choline, as well as other correlated one-carbon metabolism–related nutrients, may protect against the development of pancreatic cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J.-M. Yuan
Development of methodology: J.-M. Yuan
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W.-P. Koh, J.-M. Yuan
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.Y. Huang, L.M. Butler, R. Wang, A. Jin, J.-M. Yuan
Writing, review, and/or revision of the manuscript: J.Y. Huang, L.M. Butler, W.-P. Koh, J.-M. Yuan
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Wang, W.-P. Koh, J.-M. Yuan
Study supervision: J.-M. Yuan

Acknowledgments

The authors thank Siew-Hong Low of the National University of Singapore for supervising the field work of the Singapore Chinese Health Study, and the Singapore Cancer Registry for assistance with the identification of cancer outcomes.

Grant Support

All authors received funding from NIH/NCI grants: R01 CA144034, UM1 CA182876.

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Received June 4, 2015; revised November 18, 2015; accepted December 6, 2015; published OnlineFirst December 28, 2015.

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